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     (FILE 'HOME' ENTERED AT 18:11:26 ON 30 NOV 2005)

     FILE 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
     AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHDS, BIOTECHNO,
     CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU,
     DDFB, DGENE, DISSABS, DRUGB, DRUGMONOG2, ...' ENTERED AT 18:12:08 ON 30
     NOV 2005
L1          734 S (STAUDINGER OR PHOSPHINE) AND (FARNESYL? OR PRENYL?)
L2          349 S L1 AND AZID?
L3          287 S L2 AND CELL
L4          256 DUP REM L3 (31 DUPLICATES REMOVED)
L5          251 S L4 AND (PROTEIN OR ENZYME)
L6          249 S L5 AND (PURIF? OR ISOLAT? OR SEPARAT? OR CHROMATOGRAPH?)
L7          193 S L6 AND ((PURIF? OR ISOLAT? OR SEPARAT? OR CHROMATOGRAPH?) (
L8            5 S L7 AND ((FARNESYL? OR PRENYL?) (S) (AZID?))


=> d l8 1-5 ibib abs

L8    ANSWER 1 OF 5  DGENE  COPYRIGHT 2005 The Thomson Corp on STN
ACCESSION NUMBER: ADZ88868  peptide       DGENE
TITLE:            Detecting an isoprenylated protein in a
                  cell, comprises contacting the  cell with
                  an isoprenyl azide substrate, in which an
                  azide from a  substrate is incorporated into the
                  protein, and detecting using a  Staudinger
                  reaction.
INVENTOR:         Zhao Y; Falck J R
PATENT ASSIGNEE:  (ZHAO-I)ZHAO Y.
      (FALC-I)    FALCK J R.
PATENT INFO:      US 2005106627   A1 20050519                18
APPLICATION INFO: US 2003-715329       20031117
PRIORITY INFO:    US 2003-715329       20031117
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     2005-394577 [40]
DESCRIPTION:      RC peptide SEQ ID NO:1.
AN    ADZ88868  peptide       DGENE
AB    The invention relates to a method (M1) for detecting a first
      isoprenylated protein in a cell. (M1) comprises: (a)
      obtaining a  synthetic isoprenyl azide substrate of a
      protein in the cell; (b)  contacting the cell
      under conditions, where the cell takes up and  incorporates
      into the protein an azide from the substrate; and (c)
      detecting the first protein from proteins produced by the
      cell with a  phosphine capture reagent by the
      Staudinger reaction. Also described: (1) labeling (M2) a
      protein in a cell comprising preparing a synthetic
      substrate of the protein comprising at least a first
      azide, and  contacting the cell under conditions where
      the synthetic substrate is  taken up and incorporated into the
      protein and the protein is labeled  with the first
      azide; and (2) a compound (C1) having the molecular  formula (I),
```

(II) or (III). (M1) is useful for detecting at least a first isoprenylated **protein** such as Ras, in a **cell**. (M2) and C1 are useful for labeling a **protein** in a **cell**. (M1) enables detection and **isolation** of isoprenylated proteins with high yield, high specificity and low contamination without harsh treatment of proteins. (M1) allows uniformly chemoselective enrichment of proteins containing an **azide** group using **phosphine** capture reagents. (M1) allows efficient analysis of dynamic **farnesylation** modification. (M1) enables identification and quantification of low-abundant **farnesylated** proteins. (M1) is suitable for the characterization of biological functions and identification of **farnesylated protein** targets in response to drugs. The present sequence represents an RC peptide, which is a CAAX-box containing peptide used in an example from the present invention to determine whether **farnesyltransferase** can used FPP **azide** as a substrate for **protein farnesylation**.

L8 ANSWER 2 OF 5 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004363657 EMBASE
TITLE: A tagging-via-substrate technology for detection and proteomics of **farnesylated** proteins.
AUTHOR: Kho Y.; Kim S.C.; Jiang C.; Barma D.; Kwon S.W.; Cheng J.; Jaunbergs J.; Weinbaum C.; Tamanoi F.; Falck J.; Zhao Y.
CORPORATE SOURCE: Y. Zhao, Department of Biochemistry, Univ. Texas Southwestern Med. Ctr., Dallas, TX 75390-9038, United States. yzhao@biochem.swmed.edu
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (24 Aug 2004) Vol. 101, No. 34, pp. 12479-12484.
Refs: 34
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040924
Last Updated on STN: 20040924

AB A recently developed proteomics strategy, designated tagging-via-substrate (TAS) approach, is described for the detection and proteomic analysis of **farnesylated** proteins. TAS technology involves metabolic incorporation of a synthetic **azido-farnesyl** analog and chemoselective derivatization of **azido-farnesyl** -modified proteins by an elegant version of **Staudinger** reaction, pioneered by the Bertozzi group, using a biotinylated **phosphine** capture reagent. The resulting **protein** conjugates can be specifically detected and/or affinity-**purified** by streptavidin-linked horseradish peroxidase or agarose beads, respectively. Thus, the technology enables global profiling of **farnesylated** proteins by enriching **farnesylated** proteins and reducing the complexity of **farnesylation** subproteome. **Azido-farnesylated** proteins maintain the properties of **protein farnesylation**, including promoting membrane association, Ras-dependent mitogen-activated **protein** kinase kinase activation, and inhibition of lovastatin-induced apoptosis. A proteomic analysis of **farnesylated** proteins by TAS technology revealed 18 **farnesylated** proteins, including those with potentially novel **farnesylation** motifs, suggesting that future use of this method is likely to yield novel insight into **protein farnesylation** . TAS technology can be extended to other posttranslational modifications, such as geranylgeranylation and myristoylation, thus providing powerful tools for detection, quantification, and proteomic

analysis of posttranslationally modified proteins.

L8 ANSWER 3 OF 5 FEDRIP COPYRIGHT 2005 NTIS on STN
ACCESSION NUMBER: 2005:185640 FEDRIP
NUMBER OF REPORT: CRISP 1R21CA107943-01
RESEARCH TITLE: A Novel Proteomics Technology for **Protein Farnesylation**
STAFF: Principal Investigator: ZHAO, YINGMING; YZHAO@BIOCHEM.SWMED.EDU, UNIVERSITY OF TEXAS SW MED CTR, 5323 HARRY HINES BLVD, DALLAS, TX 75390
PERFORMING ORGN: UNIVERSITY OF TEXAS SW MED CTR/DALLAS, DALLAS, TEXAS
SUPPORTING ORGN: Supported By: NATIONAL CANCER INSTITUTE
PROJECT START DATE: 2006 (/07/04)
FISCAL YEAR: 2004
ESTD COMPLETION DATE: 2005 (/31/05)
FUNDING: New Award (Type 1)
FILE SEGMENT: National Institutes of Health
SUM DESCRIPTION (provided by applicant): The identification and characterization of changes in the level of **farnesylated** proteins in response to the treatment of **farnesyltransferase** inhibitors (FTIs), a newly introduced family of antitumor agents currently undergoing clinical evaluation, represents a major scientific challenge. Extant proteomics methods are limited to quantifying a few thousands of the most abundant proteins and, therefore, are unsuitable for the targeted profiling of less abundant **farnesylated** expression. The major goal of this application is to develop and validate a powerful technology, Tagging via **Azido** Substrate (TAS), for the efficient **isolation** of **farnesylated** proteins. This technology will then be applied to the proteomics analysis of **farnesylated** proteins in physiologically relevant models. The TAS technology involves the introduction of a synthetic **azide**-modified **farnesyl** substrate, either **farnesyl azide** diphosphate (FPP-**azide**) or **farnesyl azide** alcohol (F-**azide**-OH), which replaces the natural substrate during cellular **protein farnesylation**. The resulting **farnesyl**-**azide** (F-**azide**)-modified proteins will be affinity-**purified** through an **azide**-specific conjugation reaction (**Staudinger** reaction) using a **phosphine** capture reagent linked to photo-cleavable beads, which can then be released by UV light-induced photo-cleavage. Since affinity **purification** relies on covalent bonding resulting from a specific conjugation reaction between an **azide** and **phosphine** capture reagent, other proteins without the F-**azide** modification can be effectively removed by thorough washing. Thus, the TAS technology will allow **farnesylated** proteins to be **isolated** with high yield, high specificity, and low contamination. The initial focus of the R21 portion of this proposal is on the development of TAS technology and its application to the **isolation** of **farnesylated** proteins. These studies will be extended subsequently to geranylgeranylated proteins. The R33 proposal will aim at applications of the TAS technology to the identification of novel FTI targets. These studies will provide fundamental information for the understanding of molecular mechanisms of FTI functions and are likely to identify novel targets for antitumor drug design.

L8 ANSWER 4 OF 5 IFIPAT COPYRIGHT 2005 IFI on STN
AN 10867911 IFIPAT;IFIUDB;IFICDB
TITLE: METHODS AND COMPOSITIONS FOR TAGGING VIA **AZIDO** SUBSTRATES
INVENTOR(S): Falck; John R., University Park, TX, US
Zhao; Yingming, Dallas, TX, US
PATENT ASSIGNEE(S): Unassigned
AGENT: FULBRIGHT & JAWORSKI L.L.P., 600 CONGRESS AVE., SUITE 2400, AUSTIN, TX, 78701, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2005106627	A1	20050519
APPLICATION INFORMATION:	US 2003-715329		20031117
FAMILY INFORMATION:	US 2005106627		20050519

DOCUMENT TYPE: Utility
Patent Application - First Publication
FILE SEGMENT: CHEMICAL
APPLICATION

GOVERNMENT INTEREST:
The government may own rights in the invention pursuant to grant number CA 85146 and GM 31278 from the NIH.

NUMBER OF CLAIMS: 32 4 Figure(s).
DESCRIPTION OF FIGURES:
FIG. 1. Schematic representation of one embodiment of the TAS technology for solid-phase-**isolation** of **azide**-labeled **farnesylated** proteins. (A) **Farnesyltransferase**-catalyzed enzymatic reaction using FPP or FPP **azide** as a substrate. (B) Chemical structures of natural FPP, and a FPP-**azide** and the corresponding alcohols, F-OH (farnesol) and F-**azide**-OH. (C) A **Staudinger** conjugation reaction between a **phosphine** and an **azide**-containing molecule. (D) The structure of a biotinylated **phosphine** capture reagent and its reaction product with an **azido-farnesylated protein** . (E) Experimental procedure for the **isolation** of F-**azide** modified proteins. **Protein** i and ii, unmodified proteins; ***protein*** iii, a **protein** modified by natural **farnesyl** group; and **protein** iv, F-**azide** modified proteins. Only F-***azide*** modified **protein** iv is **purified** and released by UV light-induced photocleavage.
FIG. 2. FPP **azide** is a substrate for in vitro **farnesylation** reaction. MALDI-TOF mass spectra of RC peptide before (A) and after (B) in vitro **farnesylation** reaction. One hundred pmol RCpeptide (KKFFCAIS) were mixed with 3000 pmol FPP **azide** and 35 pmol FTase in 7 mu l reaction buffer and incubated at 30 degrees C. for 10 h. The F-**azide** modified RC-peptide was confirmed by mass spectrometry (m/z, 1191).
FIG. 3. Detection of F-**azide** modified Ras and HDJ-2 by mobilityshift assay. Mobility-shift assay of Ras (A) and HDJ-2 (B). COS1 cells were labeled with the indicated compounds for 24 h, the **cell** lysate were resolved in SDS-PAGE and probed using anti-Ras and anti-HDJ-2 antibodies, respectively. The letters, "u" and "p", indicate the unmodified and isoprenylated forms of the **protein**, respectively.
FIG. 4. Global detection of F-**azide** modified proteins by Western blotting analysis. COS cells were labeled with DMSO, or FPP N3 with or without lovastatin, as indicated, for 24 h. The **protein** lysates from the cells were precipitated by acetone/TCA method, redissolved in a buffer containing 2% SDS and PBS. The resulting solution was conjugated to biotinylated **phosphine** capture reagents or not, precipitated by acetone/TCA method again. The **protein** pellet was redissolved in 1xSDS sample buffer, resolved in SDS-PAGE, and detected by Western blotting analysis using streptavidin-conjugated HRP with or without biotin.

AB The invention provides methods and compositions for **azide** tagging of biomolecules. In one embodiment of the invention, proteins are tagged by metabolic incorporation of **prenylated azido** -analog substrates. Examples of such analogs are **azido** famesyl diphosphate and **azido** famesyl alcohol. The **azido** moiety in the resulting modified proteins provides an affinity tag, which can be chemoselectively captured by an **azide**-specific conjugation reaction, such as the **Staudinger** reaction, using a **phosphine** capture reagent. When the capture agent is biotinylated, the resulting conjugates can be detected and affinity-**purified** by streptavidin-linked-HRP and streptavidinconjugated agarose beads, respectively. The invention allows detection and

isolation of proteins with high yield, high specificity, and low contamination without harsh treatment of proteins.

CLMN 32 4 Figure(s).

FIG. 1. Schematic representation of one embodiment of the TAS technology for solid-phase-**isolation** of **azide**-labeled **farnesylated** proteins. (A) **Farnesyltransferase** -catalyzed enzymatic reaction using FPP or FPP **azide** as a substrate. (B) Chemical structures of natural FPP, and a FPP-**azide** and the corresponding alcohols, F-OH (farnesol) and F-**azide**-OH. (C) A **Staudinger** conjugation reaction between a **phosphine** and an **azide**-containing molecule. (D) The structure of a biotinylated **phosphine** capture reagent and its reaction product with an **azido-farnesylated protein**. (E) Experimental procedure for the **isolation** of F-**azide** modified proteins. **Protein** i and ii, unmodified proteins; **protein** iii, a **protein** modified by natural **farnesyl** group; and **protein** iv, F-**azide** modified proteins. Only F-**azide** modified **protein** iv is **purified** and released by UV light-induced photocleavage.

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L8 ANSWER 5 OF 5 USPATFULL on STN

ACCESSION NUMBER: 2004:267776 USPATFULL

TITLE: Methods and compositions for peptide and **protein** labeling

INVENTOR(S): Ting, Alice Y., Allston, MA, UNITED STATES

PATENT ASSIGNEE(S): Massachusetts Institute of Technology, Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2004209317	A1	20041021	
APPLICATION INFO.:	US 2004-754911	A1	20040109	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-438939P	20030109 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: Maria A. Trevisan, Wolf, Greenfield & Sacks, P.C., 600

Atlantic Avenue, Boston, MA, 02210
NUMBER OF CLAIMS: 69
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 2729
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods of use thereof for labeling peptide and proteins in vitro or in vivo. The methods described herein employ biotin ligase mutants and biotin analogs recognized by such mutants.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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=> s l8

L9 1 L8

=> d l9 ibib abs

L9 ANSWER 1 OF 1 FEDRIP COPYRIGHT 2005 NTIS on STN
ACCESSION NUMBER: 2005:185640 FEDRIP
NUMBER OF REPORT: CRISP 1R21CA107943-01
RESEARCH TITLE: A Novel Proteomics Technology for **Protein Farnesylation**
STAFF: Principal Investigator: ZHAO, YINGMING; YZHAO@BIOCHEM.SWMED.EDU, UNIVERSITY OF TEXAS SW MED CTR, 5323 HARRY HINES BLVD, DALLAS, TX 75390
PERFORMING ORGN: UNIVERSITY OF TEXAS SW MED CTR/DALLAS, DALLAS, TEXAS
SUPPORTING ORGN: Supported By: NATIONAL CANCER INSTITUTE
PROJECT START DATE: 2006 (/07/04)
FISCAL YEAR: 2004
ESTD COMPLETION DATE: 2005 (/31/05)
FUNDING: New Award (Type 1)
FILE SEGMENT: National Institutes of Health
SUM DESCRIPTION (provided by applicant): The identification and characterization of changes in the level of **farnesylated** proteins in response to the treatment of **farnesyltransferase** inhibitors (FTIs), a newly introduced family of antitumor agents currently undergoing clinical evaluation, represents a major scientific challenge. Extant proteomics methods are limited to quantifying a few thousands of the most abundant proteins and, therefore, are unsuitable for the targeted profiling of less abundant **farnesylated** expression. The major goal of this application is to develop and validate a powerful technology, Tagging via **Azido** Substrate (TAS), for the efficient **isolation** of **farnesylated** proteins. This technology will then be applied to the proteomics analysis of **farnesylated** proteins in physiologically relevant models. The TAS technology involves the introduction of a synthetic **azide**-modified **farnesyl** substrate, either **farnesyl azide** diphosphate (FPP-**azide**) or **farnesyl azide** alcohol (F-**azide**-OH), which replaces the natural substrate during cellular **protein farnesylation**. The resulting **farnesyl-azide** (F-**azide**)-modified proteins will be affinity-**purified** through an **azide**-specific conjugation reaction (**Staudinger** reaction) using a **phosphine** capture reagent linked to photo-cleavable beads, which can then be released by UV light-induced photo-cleavage. Since affinity **purification** relies on covalent bonding resulting from a specific conjugation reaction between an **azide** and **phosphine** capture reagent, other proteins

without the F-**azide** modification can be effectively removed by thorough washing. Thus, the TAS technology will allow **farnesylated** proteins to be **isolated** with high yield, high specificity, and low contamination. The initial focus of the R21 portion of this proposal is on the development of TAS technology and its application to the **isolation** of **farnesylated** proteins. These studies will be extended subsequently to geranylgeranylated proteins. The R33 proposal will aim at applications of the TAS technology to the identification of novel FTI targets. These studies will provide fundamental information for the understanding of molecular mechanisms of FTI functions and are likely to identify novel targets for antitumor drug design.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	61.73	318.71

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